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Vernakalant does not alter early repolarization or contractility in normal and electrically remodelled atria

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Aims

Besides the inhibition of the sodium inward current, vernakalant also inhibits the ultra rapid rectifier (I_{Kur}) and transient outward current (I_{to}). Inhibition of these currents increases contractility in canine atrial myocytes and goat atria. We investigated the effect of vernakalant on early repolarization and contractility in normal and electrically remodelled atria.

Methods and results

Goats were implanted a pressure catheter, piezoelectric crystals, and electrodes to obtain atrial contractility and effective refractory period (ERP). The active component in pressure distance loops was used to compute the atrial work index (AWI). Experiments were performed in normal and electrically remodelled atria at clinically relevant plasma levels of vernakalant. As a positive control, the I_{to}/I_{Kur} blocker AVE0118 was investigated. Monophasic action potentials were recorded in anaesthetized goats and in explanted hearts to determine changes in action potential morphology. Vernakalant did not affect atrial work loops during sinus rhythm. Likewise vernakalant did not increase atrial fractional shortening or AWI during pacing with fixed heart rate and AV-delay. In contrast, AVE0118 did increase AWI, with a positive force frequency relation. Both in normal and remodelled atria, vernakalant strongly increased ERP but did not prolong early repolarization.

Conclusion

In goat atria, vernakalant does not have an atrial positive inotropic effect and does not affect early repolarization. At high rates vernakalant may even have a negative inotropic effect.

Keywords

Vernakalant • Atrial fibrillation • Ion current • Action potential • Repolarization • Remodeling • Contractility

Introduction

Patients with atrial fibrillation (AF) have reduced exercise tolerance and a five-fold increase in stroke risk. Electrical or pharmacological cardioversion of AF is often performed to improve symptoms caused by rapid and irregular ventricular activations and restore the active atrial contribution to ventricular filling. However, the atria need to recover from low atrial contractility after termination of AF. This transient loss of contractility contributes to decreased exercise tolerance and might lead to thromboembolic events after termination of AF. The recovery of atrial contractility is dependent on factors such as underlying heart disease, atrial diameter, and the duration of AF. In the goat model a close correlation between AF-induced electrical remodelling and loss of atrial contractility after several days of AF (dAF) has been described in. Therefore, normalizing electrophysiological properties in the electrically remodelled heart could be a therapeutic target to enhance atrial contractility and thereby increase exercise tolerance and reduce stroke risk in the first days post-cardioversion.

In canine atrial myocytes changes in action potential morphology affect atrial cellular contractility. Specifically, increased plateau amplitude or prolongation of plateau duration facilitates Ca^{2+} entry through the reverse mode of the Na^{+}/Ca^{2+} exchanger (NCX) and leads to more pronounced cell shortening. In atrial myocytes the transient outward current (I_{to}) and the ultra rapid rectifier (I_{Kur}) largely

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What’s new?

- Vernakalant does not have an atrial positive inotropic effect in normal or electrical remodelled atria during sinus rhythm.
- Vernakalant may have negative inotropic effects at high frequencies.
- The lack of inhibition of initial repolarization prevents vernakalant to increase atrial contractility.

determine the early repolarization. Inhibition of these currents delays early repolarization and causes a positive inotropic effect in human atrial trabeculae and improved atrial contractility in the goat.6,7

Vernakalant has been available in European countries since 2010 for acute cardioversion of AF.8 Vernakalant inhibits both depolarizing sodium current and repolarizing potassium currents including the transient outward current (Ito) and the ultra rapid rectifier (Iakr).9 Moreover, Torp-Pederson et al.10,11 showed that a 90-day oral treatment of vernakalant can be safely used in haemodynamically stable AF patients. These properties of vernakalant might allow a positive inotropic treatment strategy after cardioversion. The goal of this study is to explore the possible atrial positive inotropic effect of vernakalant.

Methods

Animal model

The research protocol was approved by the local ethics committee and complied with the European directives concerning the use of animals for scientific purposes. Ten goats with a body weight of 60 ± 5 kg were included into this study. The goats were anaesthetized (i.v. sufentanyl 6 μg/kg/h and midazolam 0.8 mg/kg/h) and the thorax was opened using a left-sided approach. To determine local contractility, piezoelectric crystals were placed on the right and left appendage (Figure 1A) and left ventricular free wall for segment length recordings. Additionally, on both atria and the left ventricle a bipolar electrode was fixated, ensuring that the site of electrode implantation would not interfere with the free movement of the segments. After a 2-week recovery period an additional trans-venous 5Fr Micro-Tip pressure catheter (Millar Inc., Houston, Texas, USA) was chronically implanted in the right atrium. To prevent thrombus formation, the goats were anti-coagulated with a daily dose of nadroparin (2850 IU SC, GlaxoSmithKline, Zeist, The Netherlands).

Study protocol

Three days after pressure catheter implantation, atrial contractility and electrophysiology (EP) were investigated in awake goats. The sequence of experiments was consistent for each goat and followed the study protocol presented in Figure 1B. The first experiment (control, CTL) was performed before any episode of AF was induced. The second experiment was performed after 2dAF. AF was initiated and maintained by atrial burst pacing for as described previously in.11 Subsequently, after >5 days of recovery from AF, AF was reinitiated for another 2 days to perform a positive control experiment with AVE0118. Finally, after full recovery AF was reinduced again for 2 days for a final open chest experiment under anaesthesia (i.v. sufentanyl 6 μg/kg/h and midazolam 0.8 mg/kg/h). Based on the work of Garratt et al. no residual effect of the previous AF episodes on AF stability is expected on the atria.2,12

Drug administration protocols

We investigated two different stable plasma levels of vernakalant, about 3 and 5 μg/ml comparable to clinical relevant concentrations. The first and lower dose (LD) was reached by a 15-min loading infusion at 0.25 mg/kg/min followed by a maintenance infusion of 0.13 mg/kg/min. The higher dose (HD) was reached after 15 min loading at 0.30 mg/kg/min followed by a maintenance dose of 0.22 mg/kg/min. All contractility and EP measurements were performed 15 min after start of the maintenance infusion for both dose levels.

The atrial positive inotropic compound AVE0118 was infused at a rate of 1.0 mg/kg/h. Time between experiments allowed full washout (>5 half lives) of both vernakalant and AVE0118.

Haemodynamic and electrophysiological measurements

Measurements were performed at baseline (saline infusion, BL) and the two different dosages of vernakalant (LD, HD) or in the presence of AVE0118. At each of these conditions the atrial effective refractory period (ERP) was measured and contractility recorded during sinus rhythm and during right atrial (RA) pacing at different basic cycle lengths (BCLs). ERP was measured with a S1S2 protocol, using a pacing output at four times above threshold.

Contractility recordings were performed during atrial and ventricular pacing with a fixed delay from atrium to ventricle (150 ms), about 30 ms shorter than the intrinsic AV-conduction. In previous goat studies11 we have observed a shift in the Wenckebach cycle length after vernakalant infusion. As the occurrence of AV block dramatically alters pre- and after-load conditions of the atrium, we performed Segment lengths, RA pressures (Pra), and electrograms were recorded for 20 s (eight-bit AD conversion, 250 Hz sampling frequency, Sonometrics corporation, London, Ontario, Canada) (Figure 1C).

During the final open chest experiment the effect of vernakalant on the morphology of the atrial action potential duration (APD) was determined using a Franz catheter (7F-110 cm–2 mm, Sachs Elektronik, Germany). Monophasic action potentials (MAPs) were recorded on the epicardial RA wall at a 1kHz sampling frequency (Pmaq, Maastricht instruments, The Netherlands).

Haemodynamic analysis

The atrial haemodynamic cycle consists of a passive and an active component. The active contraction is delineated by atrial shortening and an atrial pressure rise (Figure 1C). The active phase ends when the volume has returned to its initial value (Figure 1D). This point is followed by ventricular contraction, leading to passive pressure, and distance changes. Haemodynamic parameters of the active contraction, such as diastolic distension, fractional shortening (FS), and developed RA pressure (AP), were manually determined offline in 20 s recordings (SonoSOFT 3.3.52, Sonometrics corp, London, Ontario, Canada) as described previously in.7 In the same recordings the atrial work index (AWI) was derived from the pressure-distance loops (Figure 1D). Custom-made analysis software (MATLAB 8.1, The Mathworks, Inc., Natick, Massachusetts, USA) was used to calculate AWI as the area under the curve in the active component of the loop (Figure 1C). Only signals recorded during the resting phase of the respiratory cycle were analysed.

Optical mapping

In seven goats (57 ± 9 kg) electrodes were implanted on the pericardium at the site of the left atrium that allowed trans-pericardial stimulation. Optical mapping was performed after 3 weeks of AF induction. Hearts were excised under anaesthesia (sufentanyl 6 μg/kg/h and midazolam 0.8
mg/kg/h, i.v.) and perfused with Krebs-Henseleit solution and prepared for optical mapping as previously described in detail. In brief, the hearts were loaded with the voltage sensitive dye di-4-ANEPPS. Fluorescence movies of a 2×2 cm area at the site of the crista terminalis were recorded with a 100×100 pixel CCD camera (Micam Ultima, Brainvision inc., Tokyo, Japan). The excitation-contraction uncoupler blebbistatin (10 μM) was used to prevent movement artifacts in the recorded optical potentials. Optical action potentials were recorded at a cycle length of 300 ms pacing in the presence of 0, 10, and 30 μM of vernakalant.

Statistics
Data were analysed using IBM SPSS statistics version 19.0.0. Parameters were tested with linear mixed effects model using a diagonal covariance structure with drug, dose, pacing cycle length, and/or stage of remodelling taken as fixed variables and animal identification taken as random variable. Results are reported as estimated mean ± SEM. Statistical significance was taken as \( P < 0.05 \).

Results
Haemodynamics effects of vernakalant during sinus rhythm and pacing
2dAF strongly reduced RA contractility. During sinus rhythm the AVI declined from 4.7 ± 1.8 at CTL to 1.1 ± 0.7 mm/mmHg at 2dAF. Infusion of vernakalant shifted the atrial work loop to the right in
both control and 2dAF experiments (Figure 2). Despite this increase in preload during sinus rhythm, vernakalant restored or increased AWI neither in control nor in 2dAF experiments.

To rule out possible AV-delay dependent effects, we performed pacing with fixed atrium to ventricle delay. Haemodynamic parameters, such as end-diastolic segment lengths, FS and ΔP are summarized in Table 1. FS in CTL experiments was comparable between both atria. Vernakalant did not significantly change FS and ΔP. In correspondence with the AWI, FS decreased in both atria after 2dAF. Also under these conditions, vernakalant did not increase or restore FS in either atrium. Left ventricular FS was not affected by vernakalant (data not shown).

To explore possible rate dependent effects we also investigated the AWI at 450, 350, and 250ms BCL in CTL and in 2dAF experiments (Figure 3A). As during sinus rhythm, AWI was strongly reduced in 2dAF experiments. Vernakalant did not affect AWI in either control or in 2dAF goats.

**Figure 2** (A) Representative recordings of pressure distance loops during sinus rhythm in control and 2dAF experiments. Despite a rightward shift apparent for both experiments, no increase in AWI can be appreciated. (B) All data AWI during sinus rhythm in control and 2dAF experiments. Control experiments are depicted in green and 2dAF experiments are depicted in red. 2dAF significantly reduced AWI but vernakalant did not affect AWI in either control or in 2dAF goats.

**Table 1** Atrial haemodynamics

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>2dAF</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Low dose</td>
</tr>
<tr>
<td>Diastolic length (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left atrium</td>
<td>27.9 ± 2.3*</td>
<td>30.5 ± 2.3**</td>
</tr>
<tr>
<td>Right atrium</td>
<td>19.1 ± 1.7**</td>
<td>20.1 ± 1.7*</td>
</tr>
<tr>
<td>FS</td>
<td>0.21 ± 0.04*</td>
<td>0.22 ± 0.04**</td>
</tr>
<tr>
<td>Left atrium</td>
<td>0.22 ± 0.02</td>
<td>0.17 ± 0.02*</td>
</tr>
<tr>
<td>Right atrium</td>
<td>1.3 ± 0.4</td>
<td>1.2 ± 0.4</td>
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Effect of vernakalant on average atrial haemodynamic characteristics determined in 20 s recording. Cardiac rhythm was controlled by RA pacing at 450 ms with a fixed AV-delay of 150 ms. Vernakalant vs. control.

*P < 0.05; **P < 0.01; 2dAF vs. control; #P < 0.05
Figure 3 (A) AWI measured during pacing with a fixed AV-delay (150 ms) at three different cycle lengths (450, 350, and 250 ms BCL). No significant effects occurred at 450 and 350 ms but a drop in AWI occurred at 250 ms cycle length pacing. (B) ERPs measured in the right atrium. At all cycle lengths the ERP increased in the presence of vernakalant. Vernakalant vs. BL; **P < 0.01; ***P < 0.001. HD vs. BL; *P < 0.05.
and 350 ms, but at the shortest cycle length, AWI significantly decreased at the highest dose. Revealing a use-dependent effect on the atrial contractility.

**Monophasic APD and excitability**

We hypothesized that delaying ‘early’ repolarization will lead to an increase in both ERP and AWI. As described earlier, no positive inotropic effect of vernakalant was found in the awake goats in different conditions. Yet, vernakalant strongly increased ERP in a dose-dependent manner. The degree of increase was comparable in both CTL and 2dAF experiments as can be appreciated in Figure 3B. Therefore, the lack of positive inotropic effect could not be explained by an inadequate dose that did not affect atrial electrophysiological properties.

To further explore the effects of vernakalant on early repolarization, we performed ERP and MAP measurements in a small subset \((n = 6)\) in the above described goats. These experiments were performed after 2dAF. APD\(_{30}\), APD\(_{75}\), and the plateau potential were not affected by vernakalant while ERP was significantly increased (Figure 4). The prolongation of ERP without APD prolongation implies a reduction in excitability. Therefore we also explored the effect on pacing threshold and interatrial conduction time. Pacing thresholds were increased from 0.95 ± 0.12 mA at baseline to 1.09 ± 0.17 mA and 1.21 ± 0.19 mA \((P = 0.041)\), LD and HD respectively. Furthermore the interatrial conduction time prolonged from 73 ± 7 ms at baseline to 97 ± 17 and 105 ± 17 ms \((P < 0.001)\), LD and HD, respectively.

We also evaluated action potential morphologies with high-resolution optical mapping \((n = 7)\) of the RA wall. In agreement with the earlier findings, vernakalant did not change the action potential morphology (Figure 5). Only a minor prolongation \((+4.3 ± 5.7 ms)\) of APD\(_{20}\) was observed at a concentration of 30 \(\mu M\).

**Positive inotropic effect of AVE0118**

In six awake goats, we also investigated AVE0118 after 2dAF. Dosage was chosen based on historical data to achieve comparable ERP prolongations to vernakalant. Figure 6 shows that both drugs produced comparable and significant prolongations of ERP. AVE0118 displayed a moderate frequency-dependent increase in AWI. At a BCL of 450 ms AVE0118 did not increase AWI but it did increase AWI from 4.6 ± 1.9 to 6.6 ± 4.4 mm Hg and from 8.7 ± 4.4 to 13.5 ± 6 mm Hg at pacing intervals of 350 and 250 ms, respectively. In contrast, vernakalant exhibited a negative frequency-dependent relationship that became significant at 250 ms BCL.
Figure 5  Atrial action potentials recordings after 3 weeks of AF using optical mapping. Isolated atria were perfused with Krebs-Henseleit solution only and with two concentrations of vernakalant. (A) Illustration of the isolated goat atria with the recorded location indicated on the right atria by a black rectangle. (B) The three panels show isochronal maps with different concentrations of vernakalant during pacing at 300 ms BCL. The lower panel illustrates three representative normalized (to maximal amplitude) and superimposed optical action potentials, illustrating a lack of effect on APD and morphology. (C) Effect of vernakalant on the APD at ADP20, APD30 and APD80 in the seven individual atrial preparations. Vernakalant vs. baseline; * P < 0.05

Figure 6  Left. Effect of vernakalant and AVE0118 on atrial refractory period in awake goats. The effect was measured at three different cycle lengths. Dosage of AVE0118 was targeted to result in a comparable increase of ERP. Right. The effect of vernakalant and AVE0118 on AWI. AVE0118 showed a moderate increase in AWI with the largest effect at 250 ms. In contrast, vernakalant did not increase AWI and revealed a negative force frequency relation.
Discussion

This study investigates the effect of vernakalant on atrial contractility in normal and electrically remodelled atria. We hypothesized that the \( I_{\text{Kur}} \) inhibiting properties of vernakalant would cause an increase in calcium influx by slowing early repolarization and would therefore increase atrial contractility. However, vernakalant did not increase atrial contractility or the work index and at shorter cycle lengths even reduced atrial contractility. These effects of vernakalant were found in both normal and electrically remodelled atria despite significant prolongation in atrial refractoriness. In addition, vernakalant did not affect early repolarization of the action potential, in keeping with its lack of effect on atrial contractility.

Restoration of atrial contractility after cardioversion of AF

Reduced atrial contractility is commonly observed after spontaneous, electrical and pharmacological cardioversion of AF. This reduction in atrial contractility is associated with decreased left ventricular function and reduced exercise tolerances. After cardioversion, improvement of left ventricular function and oxygen extraction may take up to 1 month after cardioversion, illustrating a delayed improvement of exercise tolerance. Therefore, acutely restoring atrial contractility might be an attractive strategy to improve recovery from AF. However, many positive inotropic strategies (e.g. \( \beta \)-adrenergic stimulation and phosphodiesterase-III inhibitors) have proarrhythmic effects on the ventricles.

In the goat model of AF, we have previously demonstrated that the initial decrease in atrial contractility went along with atrial electrical remodelling. Electrical remodelling is characterized by shortening of the APD due to downregulation of \( I_{\text{CaL}} \) and the upregulation of the inward rectifying current \( I_{\text{K1}} \). The \( I_{\text{CaL}} \) is—besides release of Ca from the sarcoplasmatic reticulum—the most important source of intracellular free \( \text{Ca}^{2+} \) and an important determinant of atrial contraction. Indeed, Wakili et al. demonstrated that atrial tachypacing induced action potential shortening and was associated with a reduction in peak \( I_{\text{CaL}} \), reduced \( \text{Ca}^{2+} \) transients and an attenuation of cell shortening. Interestingly they demonstrated that in these cells a restoration of control-like action potentials, by action potential clamp experiments, increased \( \text{Ca}^{2+} \) transients and improved cell shortening. Furthermore pharmacological changes in action potential, by modulating acetylcholine-mediated potassium current, reveals a strong relation between APD and contractility. These observations are in agreement with our previous work where we demonstrated that action potential morphology in non-remodelled atrial myocytes, more specifically the plateau phase, is crucial for cellular contractility.

Elevation of the action potential plateau in atrial myocytes by blocking the atrial specific \( I_{\text{Kur}} \) current in combination with the transient outward current \( I_{\text{to}} \) by AVE0118 revealed a linear relationship between plateau duration and cell shortening. de Haan et al. translated this observation to a whole animal model, demonstrating that AVE0118 prolonged plateau duration and fully restored atrial work in remodelled goat atria. Given that vernakalant is a known \( I_{\text{to}} \) and \( I_{\text{Kur}} \) blocker in HEK cell expression systems, a positive inotropic effect could be expected for vernakalant. However, despite a marked increase of ERP, we did not detect any effect on AWI or FS during sinus rhythm. In fact, pacing at higher frequencies revealed a negative inotropic effect.

Why does vernakalant not increase atrial contractility?

Loss of atrial contractility and disappearance of action potential plateau after cardioversion of AF is caused by attenuated calcium transients and reduced cytosolic \( \text{Ca}^{2+} \). Specifically, the down-regulation of L-type calcium channels and up-regulation of repolarizing \( K^- \) currents are responsible for the loss of contractility and action potential plateau. Hence, increasing calcium influx through L-type calcium channels, by e.g. beta adrenergic stimulation or an L-type channel agonist, will only have a limited effect in electrically remodelled atria. Indeed, in the goat model of AF the sympathomimetic drug dobutamine or the L-type \( \text{Ca}^{2+} \) agonist BayY5959 had no or a limited effect on atrial work. Alternatively, calcium entry that occurs in the early phase of repolarization through the reverse mode of the NCX may offer a potential target. Indeed, patch clamp experiments have provided evidence that the positive inotropic effect of AVE0118 is mediated by \( \text{Ca}^{2+} \) entry through this reverse mode of the NCX during early repolarization. Based on the data found in the HEK cells one might expect a similar effect in of vernakalant on early repolarization. However, in this series of experiments, vernakalant only led to a small increase in APD as measured in the optical action potentials with a negligible effect size of only 4 ms. Moreover, vernakalant did not alter the APD or the plateau phase. This absence of an effect on the action potential plateau may in part explain why vernakalant did not possess any positive inotropic effect. Of note, Wettwer et al. observed a similar lack of effect on the plateau phase in isolated human trabeculae. Despite a small but significant prolongation of the APD20 in trabeculae of chronic AF patients, vernakalant did not increase the membrane potential during the plateau phase.

Various processes could have prevented an elevation of action potential plateau and an increase of contractility. It might be that the dosage used was not sufficient to substantially inhibit \( I_{\text{Kur}} \). In the optical mapping experiments we saw a small APD20 increase at a high concentration of vernakalant. This minor prolongation of APD20 was found at a concentration that is about three-fold higher than the therapeutic plasma concentration. Such a condition was not investigated in vivo because we aimed to explore vernakalant’s positive inotropic potential at clinically relevant plasma concentrations. Further, the degree of remodelling may have affected \( \text{Ca}^{2+} \) handling to such an extent that a positive inotropic effect through the NCX pathway could no longer be possible. However, in this case a positive inotropic effect would have occurred in healthy atria, which was not the case in our experiments. Moreover, in the same animals AVE0118 did increase atrial contractility at similar baseline conditions. Taken together, these findings indicate that the \( I_{\text{Kur}} \) inhibition that has been shown by others has a limited effect on early repolarization and thus does not increase atrial contractility.

Class I effects of vernakalant

In a previous study, we have reported that vernakalant’s main antiarrhythmic effects in the awake goat are a slowing of conduction and a refractory period prolongation due to post-repolarization refractoriness. This study shows an use-dependent effect on atrial contractility
and a decrease in excitability described by increased pacing thresholds and slowing of conduction. The use-dependent effect of vernakalant on the sodium current may explain the negative inotropic effect at faster pacing rates. These haemodynamic and electrophysiological observations in vivo, and ex vivo measurements, underscores the potent and dominant class I effects of vernakalant. This inhibition of sodium current presumably will affect NCX function. Specifically, the reduced voltage dependent sodium current will lead to a decreased cytosolic sodium concentration, lowering the driving force for Ca\(^{2+}\) influx through the NCX. Thereby limiting the potential contribution of the NCX reverse mode on atrial contractility.

**Limitations**

This animal model requires chronic implantation of electrodes and crystals. It is likely that this has caused a local inflammation and in turn affected local electrophysiological and contractile properties. In control conditions and after the infusion of AV0118 significant atrial work could clearly be measured but this does not exclude a negative effect on atrial contractility of the model itself.

Second, in patients with longstanding persistent AF, LA contractility is affected to a greater extent than the right atrium. Our study only evaluated RA work loops because of the risk of clot formation caused by chronic implantation of a pressure catheter in the left atrium. We assume that RA work is in agreement with LA work, comparable to the lack of effect on FS in both atria. Furthermore, differential effects action potential morphology are not expected as we did not detect differences in effect of vernakalant on ERP or conduction velocity between both atria a previous study.

**Conclusions**

Vernakalant does not affect atrial contractility, arguably because it does not affect early atrial repolarization. Most likely vernakalant does not significantly inhibit I\(_{\text{Kur}}\) at clinical relevant plasma levels. Our data confirm recent observations that vernakalant mainly acts by its effects on the sodium current.

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**References**